DRG® Corticosterone (Rat/Mouse) Elisa (EIA-5186)

Revised 23 May 2011 rm (Vers. 4.1) **For Veterinary Use Only**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTRODUCTION

1.1 Intended Use

The Corticosterone rat/mouse ELISA is a competitive immunoassay for the quantitative measurement of corticosterone in rat and mouse serum or plasma.

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1.2 Summary and Explanation

Corticosterone is secreted by the adrenal cortex under control of the pituitary hormone ACTH via a negative feedback mechanism. It is the most abundant circulating steroid in rats, since rodents are not able to synthesize Cortisol, the major glucocorticoid in human, as a result of lacking the enzyme C17-Hydroxylase.

Corticosterone has a wide range of activities in rodents. It regulates carbohydrate, protein and fat metabolism. It has also an influence on the hemopoietic system and reduces the total number of lymphocytes and eosinophils, but to a lesser extent than cortisol. In contrast to cortisol, corticosterone has only minimal anti-inflammatory activity.

Corticosterone level in nocturnal animals like rats exhibit a distinct circadian variation with peak values in the latter portion of the day, followed by a nadir in the morning (1) and is believed to play an important role in sleep-wake cyclus (2). This is in contrast to diurnal mammals, where peak concentrations of glucocorticoids are found in the morning. Enhanced corticosterone release by female compared to male rats under basal and stress conditions has been observed (6).

Determination of corticosterone in rats is of interest to facilities conducting neurophysiological research, to academic institutions and to pharmaceutical companies with drug research departments. Drugs that influence the endocrine system can increase or reduce corticosteroid production in the adrenal cortex. Rat serum corticosterone is therefore an ideal indicator of the side effects of a potential therapeutic agent. The same constellations of effects seen in rats are generally seen in human. Plasma corticosterone in rats is often used in connection with ACTH measurement as a stress indicator (3,4). The effects of chronic stress on the function of the hypothalamic-pituitary-adrenocortical system are age-dependent. Recent studies suggest that aging increases basal but not stress induced levels of corticosterone in the brain (5).

2 PRINCIPLE

The **Corticosterone rat/mouse ELISA** Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

An unknown amount of corticosterone present in the sample and a defined amount of corticosterone conjugated to horseradish peroxidase compete for the binding sites of corticosterone antiserum coated to the wells of a microplate. After incubation on a shaker the microplate is washed four times. A

fter addition of the substrate solution the concentration of corticosterone is inversely proportional to the optical density measured.

3 WARNINGS AND PRECAUTIONS

1. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. Do not mix reagents of different lots. Do not use expired reagents.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.
5. Avoid contact with Stop Solution. It may cause skin irritation and burns.
6. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
7. Change pipette tips between samples and reagents to avoid carry over contamination.
8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
11. Assay reagents contain Proclin against microbial growth. In case of contact with eyes or skin, flush immediately with water.
12. All reagents should be at room temperature (21-26°C) before use. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
13. TMB substrate has an irritant effect on skin and mucosa. In case of contact with skin or eyes, wash thoroughly with water. Please note that extreme temperature changes may cause spontaneous decay of the peroxide.

4 REAGENTS

4.1 Reagents provided

1. **Microtiterplate**, 12 x 8 (break apart) strips with 96 wells;
   Wells coated with polyclonal rabbit anti-corticosterone antibody.
2. **Calibrator 0,** 1 vial, 0.3 ml, ready to use
3. **Calibrator (Calibrator 1-5),** 5 vials, 0.3 ml each, ready to use;
   Concentrations: 15 – 50 – 185 – 640 – 2250 ng/ml
4. **Incubation Buffer,** 1 vial 11 ml, ready to use;
5. **Enzyme Conjugate**, 1 vial, 7 ml, ready to use;
   Corticosterone conjugated to horseradish peroxidise.
6. **Substrate Solution**, 1 vial, 22 ml, ready to use;
   contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
7. **Stop Solution**, 1 vial, 7 ml, ready to use;
   contains 2 N Hydrochloric Acid solution.
8. **Wash Solution**, 1 vial, 50 ml (10X concentrated);
   see „Preparation of Reagents“.

Note: Additional Calibrator 0 for sample dilution is available upon request.

4.2 Materials required but not provided

Centrifuge
A microtiter plate reader capable for endpoint measurement at 450 nm
Microplate mixer operating more than 600 rpm
Vortex mixer
Calibrated variable precision micropipettes (10 µl, 50 µl, 100 µl, 200 µl).
Absorbent paper
Distilled or deionized water
Timer
Semi logarithmic graph paper or software for data reduction

4.3 Reagent preparation

All reagents should be at room temperature before use.

Wash Solution:

Dilute 50 ml of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml.
The diluted Wash Solution is stable for at least 3 months at room temperature.

4.4 Storage conditions

When stored at 2°C to 8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.

5 SPECIMEN

Serum

Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C.
Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Use in assay immediately or aliquot and store samples at -20°C.
Avoid repeated freeze-thaw cycles.

Please note: The use of plasma as specimen can result in a diminished precision of this assay.

6 ASSAY PROCEDURE

6.1 General Remarks

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.

Once the test has been started, all steps should be completed without interruption.

Use new disposal plastic pipette tips for each standard and sample in order to avoid cross contamination.

Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

As a general rule the enzymatic reaction is linearly proportional to time and temperature.

For internal quality control we suggest to use the Rat Control (Fertility / Pregnancy), REF CTL-5262. For more information please contact DRG.

6.2 Assay Procedure

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense **10 µl** of each **Calibrator and Sample** with new disposable tips into appropriate wells.
3. Dispense **100 µl** of **Incubation Buffer** into each well.
4. Add **50 µl Enzyme Conjugate** into each well.
5. Incubate for **2 hours** at room temperature on a microplate mixer.
   Important Note:
   Optimal reaction in this assay is markedly dependent on shaking of the microplate!
6. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7. Add **200 µl** of **Substrate Solution** to each well.
8. Incubate without shaking for **30 minutes** in the dark.
9. Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
10. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

6.3 Calculation of results

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the calibration curve.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.

5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Conversion to SI units:
Corticosterone (ng/mL) x 2.886 = nmol/Ll

6.3.1 Example of Typical Calibrator Curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard	Absorbance Units
Calibrator 0 (0 ng/ml)	2.398
Calibrator 1 (15 ng/ml)	2.094
Calibrator 2 (50 ng/ml)	1.762
Calibrator 3 (185 ng/ml)	1.030
Calibrator 4 (640 ng/ml)	0.385
Calibrator 5 (2250 ng/ml)	0.134

7 EXPECTED NORMAL VALUES

In order to determine the normal range of serum corticosterone in rat, samples of male and female rats were collected in the morning (7.00 – 9.00 am) as well as in the late afternoon (5.00 – 6.00 pm) and analyzed using the Corticosterone rat/mouse ELISA kit. The following ranges are calculated with the results of this study.

	Range (ng/ml) Morning	Range (ng/ml) Late afternoon
Male rats ♂	3.6 – 11.4	172.6 – 245.4
Female rats ♀	53.9 – 332.1	292.5 – 819.0

In further studies serum samples of 23 mice were collected between 11.00 am and 2.00 pm und analyzed in a similar manner.

	Range (ng/ml)
Male mice ♂	47 – 159

It is recommended that each laboratory establish its own normal range since corticosterone levels can vary due to handling and sampling techniques.

8 PERFORMANCE CHARACTERISTICS

8.1 Analytical sensitivity

The lowest analytical detectable level of corticosterone that can be distinguished from the Zero Calibrator is 4.1 ng/ml at the 2SD confidence limit.

8.2 Specificity

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to corticosterone.

Steroid	% Cross reaction
Aldosterone	0.2
Cortisol	0.3
11-Deoxycorticosterone	2.4
Dehydroepiandrosterone	n.d.
Estriol	n.d.
Estradiol	n.d.
17-Hydroxyprogesterone	n.d.
Progesterone	0.7
Testosterone	n.d.
18-Hydroxydeoxycorticosterone	n.d.
Pregnolone	n.d.

n.d. = non detectable

8.3 Reproducibility

8.3.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 6 serum samples within one run. The within-assay variability is shown below:

Mean (ng/ml)	91.6	229.7	365.4	96.8	189.1	330.7
SD	7.6	13.0	15.2	6.6	7.5	9.2
CV (%)	8.3	5.7	4.2	6.8	4.0	2.8
n =	20	20	20	20	20	20

8.3.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of 3 serum samples.

Mean (ng/ml)	27.8	267.8	892.8
SD	3.5	12.9	65.7
CV (%)	12.4	4,8	7.4
n =	10	10	10

8.4 Recovery

Using the Corticosterone rat/mouse Incubation buffer three spiking solutions were prepared (A = 2000 ng/ml B = 4000 ng/ml and C = 5000 ng/ml).

A 25 µl aliquot of each solution was spiked into 475 ml of three rat sera for a spiking ratio of 1 to 19, leaving the serum matrix of the spiked samples relatively intact. All samples were then measured by the Corticosterone rat/mouse ELISA procedure. To calculate expected values 95% of the unspiked values were added to 5% of the spiking solution concentrations (50, 100 and 125 ng/ml, respectively).

Serum	Spiking Solution	Observed (0)	Expected (E)	O/E %
1	-	137	-	-
	A	201	230	87%
	B	274	330	83%
	C	317	380	83%
2	-	285	-	-
	A	391	370	106%
	B	450	470	96%
	C	533	520	103%
3	-	192	-	-
	A	303	282	107%
	B	427	382	112%
	C	497	432	115%

8.5 Linearity

Five native serum samples were assayed undiluted and diluted with the calibrator matrix.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration	(ng/ml)	382	332	300	321
Average %	Recovery	99.8	98.0	112.3	107.3
Range of	from	95.8	92.9	105.3	100.0
Recovery %	to	102.6	102.4	118.4	112.5

9 LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Drug Interferences

Until now no substances (drugs) are known influencing the measurement of rat or mouse corticosterone in serum. Lipemic and haemolysed samples can cause false results.

10 REFERENCES / LITERATURE

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Version 2011-05-10~rm